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Endothelial and potassium channel dependent modulation of noradrenergic vasoconstriction in the pig radial artery

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ABSTRACT

The localisation and function of noradrenergic perivascular innervation of the radial artery were examined in a porcine model. Through immunohistochemical techniques, we explored the possible existence of dopamine beta-hydroxylase and choline-acetyltransferase in the nerve fibres supplying the radial artery. Arterial rings suspended in organ baths were used to isometrically record tension in functional tests designed to determine the vasoconstriction response to electrical field stimulation (EFS) or exogenous noradrenaline. Morphological studies revealed the presence of noradrenergic, but not cholinergic, nerve fibres in the tunica adventitia and adventitia-media boundary of the artery wall. EFS-elicited frequency-dependent contractions ($EF_{50} = 3.37 \pm 0.19$ Hz and $E_{max} = 87.7 \pm 3.8\%$; $n = 47$) were abolished by tetrodotoxin. The contractile effect was markedly reduced by guanethidine, phentolamine and prazosin and slightly inhibited by rauwolscine, but unaltered by propranolol, atropine, bosentan or capsaicine. Endothelium removal increased EFS-evoked contractions but the addition of L-NOArg, ODQ or indomethacin had no effect. Pre-incubation with tetraethylammonium and 4-aminopyridine, but not glibenclamide, enhanced these neurogenic responses. SOD and apocynin reduced EFS-elicited responses at low frequencies. Exposure of the arterial rings to the same agents did not affect the noradrenaline concentration–response curves except for the α-adrenoceptor antagonists. These results led to the conclusions that neurogenic contractions in the pig radial artery are predominantly mediated by noradrenaline released from periarterial adrenergic nerves. This neurogenic vasoconstriction is modulated by a non-NO, non-prostanoid endothelium-dependent relaxing factor and by Ca^{2+}-activated and voltage-dependent K^+ channels.

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1. Introduction

Myocardial revascularization has demonstrated its potential as effective long-lasting treatment for ischaemic heart disease. As the key elements of coronary artery bypass grafting, the use of arterial segments such as the left internal mammary artery, which has provided spectacular clinical outcomes for bypassing the left anterior descending coronary artery, have encouraged the use of other arteries, including the radial artery. The radial artery has been used as an alternative coronary arterial autograft in patients with multiple stenoses for complete revascularization using only arterial conduits. Effectively, recently reported mid-term patency rates support the use of this vessel as the second arterial autograft after the internal mammary artery (González Santos et al., 2005; Stojnic et al., 2006).

Several studies have compared the vasoreactivity of the human internal mammary artery and radial artery (Ding et al., 2008; Mangoush et al., 2008). It has been well-established that the radial artery is much more reactive to vasoconstrictors, such as potassium, noradrenaline, 5-hydroxytriptamine, angiotensin II and endothelin-1, than the internal mammary artery (He and Yang, 1997). On the contrary, the internal mammary artery shows a significantly enhanced capacity over the radial artery for nitric oxide (NO) release or endothelium-derived hyperpolarizing factor (EDHF)-mediated hyperpolarization (He and Liu, 2001).

The vascular tone of bypass grafts is influenced by endothelial, hormonal and neural factors among others (Novella et al., 2007; Rosenfeldt et al., 1999). Endothelial and hormonal vascular regulation have been studied in the human radial artery, with particular reference to internal mammary artery function (Wei et al., 2007; Zulli et al., 2008). Although adrenergic receptors have been characterized in human radial artery (He and Yang, 1998), the neurogenic modulation of this blood vessel has been scarcely addressed. Histological studies have identified nerve fibres in the tunica adventitia of human radial artery (Barry et al., 2003) and, as far as

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we are aware, only Stojnic et al. (2006) have observed a neurogenic response to EFS (20 Hz) as the contraction of isolated human radial artery rings. The vessels used in this type of experiments are usually obtained from patients undergoing coronary artery bypass grafting. However, the multiple cardiovascular risk factors of these patients could affect the vascular reactivity of this type of arterial specimen. Given the pig is a well-established experimental model for cardiac surgery (Brann et al., 2000; Lee, 1986), we used segments of porcine radial artery in our experiments in an attempt to avoid the detrimental impact of these cardiovascular risk factors on the vasoreactivity.

The aim of this study was to analyse neurogenic contractions produced in porcine radial artery segments using a combined histological/functional approach.

2. Methods

The research protocol of this study was approved by the Ethical Committee for Animal Welfare of Hospital Universitario Ramon y Cajal. The investigation is also conformed with the Spanish Normative for the Care and Use of Laboratory Animals (R.D. 1201/2005).

2.1. Immunohistochemical studies

Radial artery segments from 8 pigs, were fixed by immersing in 4% paraformaldehyde prepared in 0.1 M sodium phosphate buffer (PB), pH 7.4 at 4 °C for 5 h, and then placed in a cryoprotective phosphate buffer solution containing 30% sucrose for 24 h at 4 °C. Specimens were frozen in CO_2 and stored at −80 °C until cross-sections of 10 μm were obtained using a cryostat microtome.

For immunohistochemistry using avidin–biotin–peroxidase complex (ABC) procedures (Hsu et al., 1981), the tissue sections were immersed in a mixture of 1% H_2O_2 and 90% methanol in distilled water and then washed in PB (3 × 10 min). Specimens were preincubated for 3 h in 10% normal goat serum in PB containing 0.3% Triton X-100 to detect dopamine-beta-hydroxylase, and in 5% bovine serum albumin and 0.25% triton X-100 to detect choline-acetyltransferase. Next, the sections were treated with rabbit anti-dopamine-beta-hydroxylase antibody (Chemicon International Inc.) diluted 1:1500 in PB, and goat anti-choline-acetyltransferase antibody (Chemicon International Inc.) diluted 1:150. Sections were then incubated for 2 h at room temperature with biotinylated anti-rabbit serum raised in goat (Chemicon International Inc.) diluted 1:400 to detect dopamine-beta-hydroxylase and with anti-goat serum raised in donkey (Chemicon International Inc.) diluted 1:400 to detect choline-acetyltransferase. Once treated with the avidin–biotin complex (ABC, Vector) diluted 1:100 for 90 min at room temperature, the resultant immunocomplex was visualized using 0.05% 3,3-diaminobenzidine and 0.001% H_2O_2 in PB.

2.2. Organ bath studies

2.2.1. Tissue preparation, dissection and mounting

Radial artery segments were obtained from 47 male cross-breed pigs (weight 40–45 kg). Arteries were cleaned of adhered tissues and cut into 3 mm-long rings (external diameter = 1.85 ± 0.07 mm, internal diameter = 0.51 ± 0.03 mm; $n = 47$). The vessel rings were transferred to 5 ml organ baths containing PSS at 37 °C and aerated with a mixture of 95% O_2 and 5% CO_2 to maintain the final pH at 7.4. The rings were mounted between two parallel L-shaped stainless steel wires. One wire was fixed to a displacement unit allowing fine adjustment of tension while the other was attached to a force transducer (Grass FT03C). The isometric tension of the vessel wall was

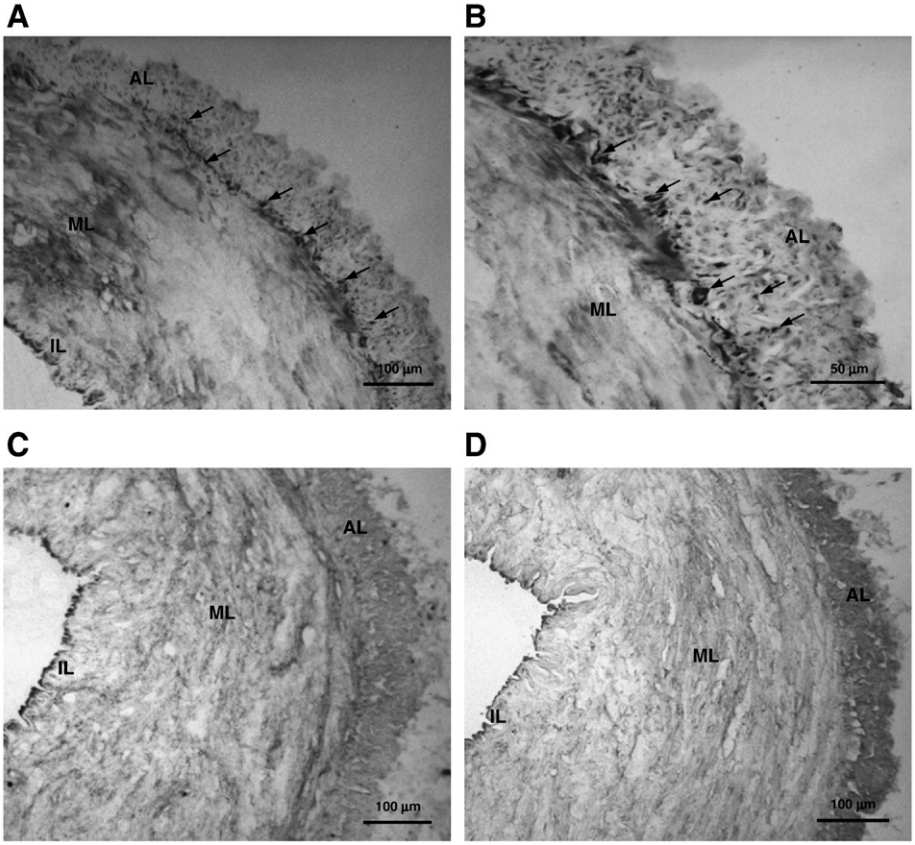

Fig. 1. Immunohistochemistry studies of porcine radial artery. Avidin–biotin–peroxidase complex counterstaining showing: (A) and (B) dopamine-beta-hydroxylase immunoreactivity, (C) choline-acetyltransferase immunoreactivity and (D) negative control for choline-acetyltransferase immunoreactivity in cross-sections of porcine radial artery. IL: intimal layer, ML: medial layer and AD: adventitial layer. Arrows indicate immunoreactivity.